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TEMPERATURE COEFFICIENTS OF RESPIRATION IN PSAMMECHINUS EGGS

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Introduction

In *Arbacia punctulata* Rubenstein and Gerard (1934) using Warburg technique, found a Q_{10} of oxygen consumption for fertilized eggs of 1.8 between 13 and 30° C., whereas unfertilized eggs had a much higher value, viz., 4.1. These remarkable findings were principally confirmed by Korr (1937), who extended the experiments (Warburg technique) and discussed the results from biochemical and physiological points of view.

On the other hand Tyler and Humason (1937), working on *Strongylocentrotus purpuratus* (Warburg technique), found no significant difference in respiration Q_{10} between fertilized and unfertilized eggs in the temperature range 5–22° C. So, for example, the Q_{10} values for 10–20° C. were 2.3 and 2.6 respectively. Similar results were reported for Dendraster, Ciona and Urechis.

In earlier investigations (Warburg technique) by Ephrussi (1933) on *Paracentrotus lividus* the same Q_{10} of oxygen uptake was found between 14.9 and 22.4° for unfertilized eggs and gastrulae, viz., $\sim$ 2–2.5. At lower temperatures there was a tendency to slightly higher Q_{10} values in the eggs. Loeb and Wasteneys (1911) using Winkler technique reported very low values for unfertilized Arbacia eggs ($Q_{10} \sim 1.3$ between 5 and 25° C.), but normal values for fertilized eggs ($Q_{10} \sim$ 2–2.5 for the range 3–25° C.).

In view of the probable significance of temperature coefficients of respiration for elucidating the different oxidative mechanisms of fertilized and of unfertilized sea-urchin eggs, it was thought of importance to investigate the matter in another species. Recently Borei (1948a) studied the respiration of eggs of *Psammechinus miliaris* before and after fertilization. Because of the facts already known about this species, it was chosen for the present investigation.

Experimental Data

The experiments have been performed with Cartesian diver micro-respiration technique, suitable for measurement of the oxygen consumption of ~100 eggs at a time. Concerning material and methods, corresponding chapters (2.1–2.3; 3.111; 3.114) in Borei (1948a) should be consulted. Diver charge type I (Borei, 1948b) was used throughout.

The temperature range was 10–21° C. The maximum temperature for normal larval development of *Psammechinus miliaris* has been studied by S. Runnström (1927), who found it to be 22° C.; the minimum temperature was found by this author to be 8° C. For measurements at lower temperatures, the cooling coil of the diver apparatus thermostat was fed with refrigerated salt-water of approximately +5° C. The desired temperature was obtained by counteracting the cooling device by operating a thermostatically controlled electric heating bulb.

In order to obtain more comparable values, measurements were only performed on the flatter part of the declining respiration curve of the unfertilized egg (cf. Borei, 1948a, Chapter 3.112.1). Thus the time of actual measurement usually ran from three to six hours after removal from the ovary. This means that the constant part of the respiration is dominant during the measurements, whereas the "rapidly declining" part characterizes the preceding 2.5–3 hours, during which the eggs were kept at 16–18° C. The eggs were, on an average, placed in the diver 2.5 hours after removal from the ovary. The diver was then immediately placed in the thermostat at the experimental temperature and left there for a half hour for temperature equilibration before starting the measurements. Usually two diver thermostats were operated simultaneously, thus allowing measurements at two different temperatures. The time schedule of the experiments may be seen from Figure 1.

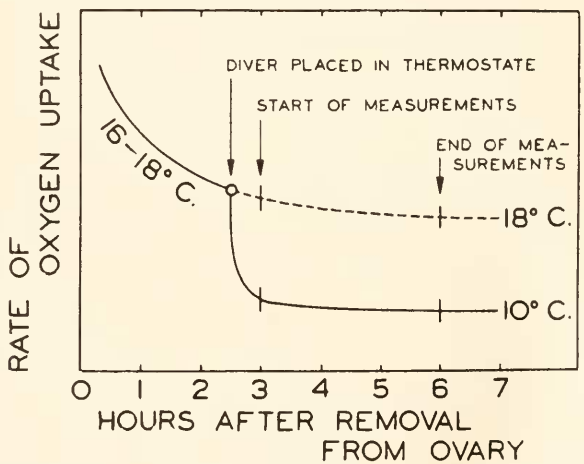

Figure 1. Average time schedule of experiments.
An actual experiment with measurements at 10° C. and a control experiment at 18° C. is assumed in the figure.

After completed diver measurements, the cells were washed out of the divers with sea-water, re-counted and then microscopically observed as to condition and fertilizability. Only those experiments were accepted in which the cells passed these post-diver measurement controls satisfactorily.

Previous authors have stated their results in rates of oxygen consumption per volume of cell matter. Great pains were taken by them to estimate the volume accurately. Probably the most correct way will be that adopted by Korr (1937): egg volume obtained by multiplying the number of eggs by the average egg volume. Technical difficulties were met, however, in estimating the exact number of eggs. In the present investigation neither the counting of the eggs nor the measurement of the average size of the eggs will give any difficulties. In view of these facts and of the great variability of the cell diameter, it has been thought more advisable, even in this investigation, to state the rate of oxygen uptake on a cell volume basis. This procedure also permits of direct comparison with the results of the previous authors.

In order to obtain a measure of the cell volume, the cell diameter was estimated of a number of eggs (> 20) from every female used, by means of a calibrated ocular micrometer (cf. Borei, 1948a, Chapter 3.114).

TABLE I

Oxygen consumption at different temperatures of unfertilized Psammechinus miliaris eggs

Volume of oxygen, measured at 0° C. and 760 mm. Hg, consumed per volume of cell matter and hour. All measurements are on egg material from S-form animals, except those marked *, which are on material of the Z-form.

Temperature (° C)	Oxygen consumption	Temperature (° C)	Oxygen consumption
10	0.024	18	0.080
	0.053		0.082
	0.069		0.084
11	0.062		0.099
	0.066		0.107*
	0.069		0.132
12	0.067		0.151*
13	0.056		0.160
	0.076	19	0.086
14	0.078		0.089
	0.083		0.140
15	0.060		0.145
	0.113		0.173
16	0.033		0.188
	0.046	20	0.065
	0.099		0.111
	0.112		0.116
17	0.061		0.126
	0.076	21	0.090
	0.088		0.122*
18	0.063		0.131
	0.065		0.172*

The oxygen consumption figures obtained are referred to 0° C. and 760 mm. Hg, in order to render them intercomparable, irrespective of the actual temperature of measurement. Corrections are introduced according to Figure 2 of Borei (1948a) for deviations from the time schedule of the above Figure 1.

The results of the experiments are given in Table I.

The average cell volume, calculated from the separate figures used for the evaluations in Table I, is 5.89×10^{-4} µl. per egg (n = 44). (Corresponding value given by Borei, 1948a, $= 5.84 \times 10^{-4}$.) The average oxygen consumption rate at 18° C. was found in the experiments (n = 10) to be 0.51×10^{-1} µl. per cell and hour. (Corresponding value given by Borei, 1948a, $= 0.53 \times 10^{-4}$.)

Discussion

Rubenstein and Gerard (1934) expressed their results according to the van't Hoff-Arrhenius equation and thought that the critical thermal increments (μ) might indicate the nature of the oxidative processes of the unfertilized and fertilized egg. This view was criticized by Korr (1937), who stressed that biological scattering and the narrow temperature limits within which respiration can be measured make the graphical evaluation of μ-values uncertain. Moreover, biological processes are governed by enzyme reactions. Such reactions have repeatedly been found not to give constant thermal increments.

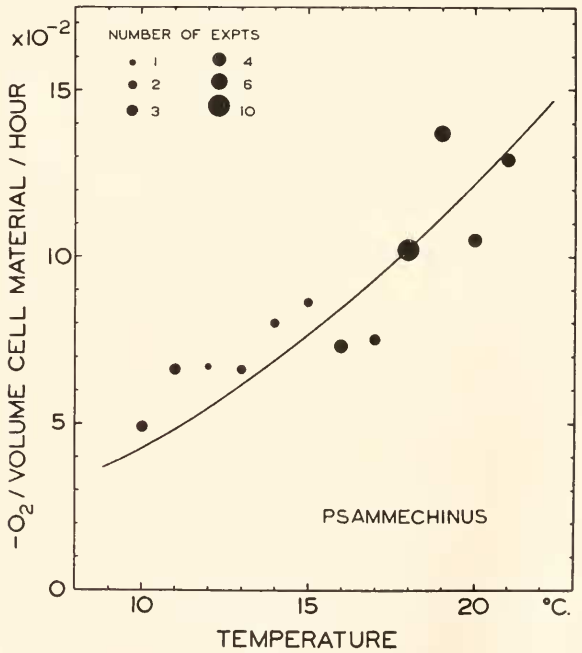

Figure 2. Oxygen consumption at different temperatures of unfertilized *Psammechinus miliaris* eggs.

Each dot is the mean value for the temperature in question. Diameter of dot indicates number of experiments. A standard curve according to Krogh (1914) is drawn in, passing through the value of 18° C.

The mass plot, recommended by Korr (1937), is difficult to interpret owing to the extent of the biological scattering. The mean of the values at each temperature gives a better representation. Figure 2 is plotted in this manner.

The figure shows that the standard curve of Krogh (1914) describes the obtained results quite well. This curve was originally obtained in basal metabolism experiments and found to be valid for a number of vertebrates. It was recently extended by Zeuthen (1947) to hold even for the respiration of a number of minute, chiefly marine invertebrate organisms. Thus Krogh's standard curve better describes the response in respiration on increase of temperature than does the van't Hoff-Arrhenius equation.

Formula (1)

For the temperature range of the present investigation the curve indicates that the relative temperature increment is very closely proportional to the relative increment in respiration. Thus it may be expressed by $(R_1/R_2)\,(t_2/t_1) = \text{const.}$, where R_1 and R_2 are the rates of respiration at the temperatures t_1 and t_2. The constant is dependent on the chosen difference between t_1 and t_2. For $t_1 - t_2 = 2°$ C., it is $= 0.93$.

In Figure 3 the results of this and previous investigations are given on a relative scale. The curve according to Formula (1) represents the material of this investigation. With this curve coincide the values of Tyler and Humason (1937) on the respiration of Strongylocentrotus eggs. On the other hand the results with Arbacia differ markedly from those on Psammechinus or Strongylocentrotus.

The temperature characteristics of the unfertilized sea-urchin egg thus represent two distinct classes: (1) the Arbacia type with high Q_{10} values, and (2) the Strongylocentrotus-Psammechinus type with Q_{10} values in close concordance with the standard curve of Krogh.

The temperature coefficients of the fertilized Psammechinus egg differ in no way from those found by previous authors for fertilized eggs of other sea-urchin species. Thus in the range 12–20° C. a $Q_{10} \sim 2$–2.5 was found.

A comparison of the Q_{10} values found in different investigations further stresses that there are two classes in respect to temperature characteristics of respiration of the unfertilized eggs (see Table II). Strongylocentrotus and Psammechinus have for both fertilized and unfertilized eggs a Q_{10} value at room temperature of approximately 2.5. The fertilized Arbacia egg shows the same value, but the value of the unfertilized egg is higher. The unfertilized egg of Paracentrotus has, at lower temperatures, a tendency in the same direction as the Arbacia egg.

Apparently Q_{10} as in most biological processes is higher at lower temperatures. The results of Tyler and Humason (1937) and of Korr (1937) seem, however, to contradict this conclusion, but the aberrations are probably to be attributed to experimental circumstances. (Lucké and co-workers, 1931, found that the Q_{10} of the permeability of the Arbacia egg to water increased with temperature.)

In the sea-urchin egg the rate of respiration is increased greatly by fertilization. This higher respiration is suppressed by cyanide, CO and other poisons of cytochrome oxidase. The oxidase in operation is an iron porphyrin, but probably not fully identical with the usual cytochrome oxidase. The respiration of the unfertilized egg was found by Runnström (1930) and Korr (1937) to be comparatively

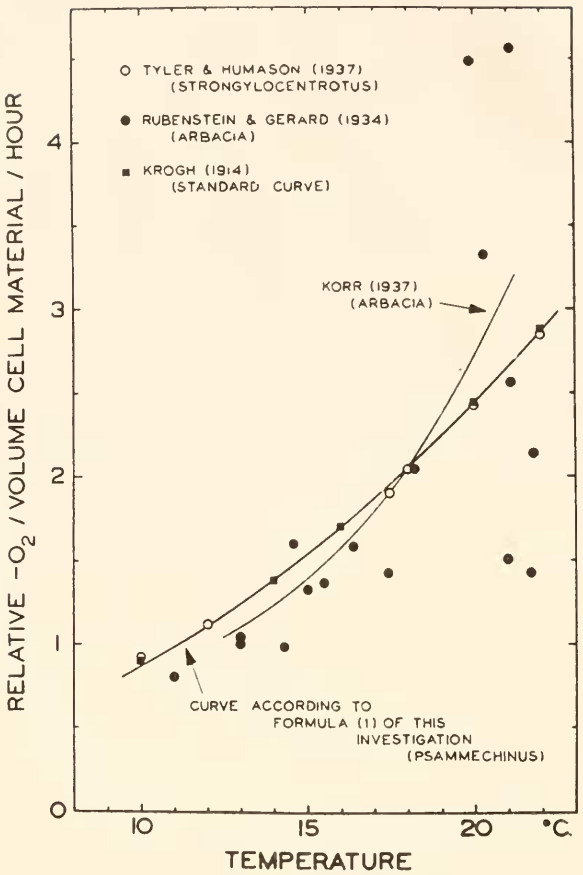

FIGURE 3. Comparison between different authors' results concerning temperature dependency of oxygen consumption in unfertilized sea-urchin eggs.

Rates of oxygen consumption given on a relative scale, putting the values at 18° C. alike. The curve for Korr's results is recalculated from this author's Figure 8.

slightly affected by said oxidase poisons. Both oxidase and dehydrogenases can be brought to work as effectively in the unfertilized egg as in the fertilized one. This permits the conclusion that neither the oxidase nor the dehydrogenases are the limiting factors in the respiration of the unfertilized egg (Runnström, 1935, 1942; cf., however, Ballentine, 1940). Runnström thinks that the difference in respiration rate between unfertilized and fertilized eggs is caused in the unfertilized egg by a lack in substrate saturation of the oxidase. Korr interprets the difference as being dependent on a factor in the oxidase part of the carrier chain, inactive or held apart in the unfertilized egg, but put into operation on fertilization. He thinks that this link might be cytochrome c and furthermore that the unfertilized egg respires over an autoxidizable, non-ferrous carrier. For a fuller review see Needham (1942) and Borei (1948a).

TABLE II

Q_{10} values of oxygen consumption of sea-urchin eggs

Author	Unfertilized eggs	Fertilized eggs
Loeb and Wasteneys (1911) †	(*Arbacia punctulata* 5–25° C. Q_{10} = 1.31)§	*Arbacia punctulata* (round 1st mitosis) 3–27° C. Q_{10} ~ 2–2.5
Rubenstein and Gerard (1934) *	*Arbacia punctulata* 13–30° C. Q_{10} = 4.1	*Arbacia punctulata* (up to 5 hours after fert.?) 13–30° C. Q_{10} = 1.8
Korr (1937) *	*Arbacia punctulata* 13–23° C. Q_{10} = 3.7 18–28 4.5	*Arbacia punctulata* (up to 5 hours after fert.?) 13–23° C. Q_{10} = 2.2, 2.8 18–28 2.5, 3.1
Ephrussi (1933) *	*Paracentrotus lividus* 10.4–22.4° C. Q_{10} = 3.85 12.9–22.4 3.28 14.9–22.4 2.18 16.75–22.4 1.88	*Paracentrotus lividus* (gastrulae) 10.4–22.4° C. Q_{10} = 2.36 12.9–22.4 2.57 14.9–22.4 2.32 16.75–22.4 1.88
Tyler (1936) *		*Strongylocentrotus purpuratus* (round 1st mitosis) 7.5–20° C. Q_{10} = 2.54 10 –20 2.30 15 –20 1.85 (25th–26th hour after fert.) 15 –20° C. Q_{10} = 1.88
Tyler and Humason (1937) *	*Strongylocentrotus purpuratus* 7.5–17° C. Q_{10} = 2.67 8 –18 2.58 10 –20 2.63 12 –22 2.54 5 –20 2.35	*Strongylocentrotus purpuratus* (a few hours after fert.) 7.5–17° C. Q_{10} = 2.79 8 –18 2.69 10 –20 2.26 12 –22 2.33 5 –20 2.62
This investigation ‡	*Psammechinus miliaris* 10–12° C. Q_{10} = 3.52 12–14 3.07 14–16 2.81 16–18 2.58 18–20 2.40 20–22 2.29	*Psammechinus miliaris* (a few hours after fert.) 12–20° C. Q_{10} ~ 2–2.5

† Winkler technique.
* Warburg experiments.
‡ Diver technique.
§ Only a single experiment.

Recent investigations on Arbacia eggs (Robbie, 1946) show that the respiration of the unfertilized egg can also be completely abolished by cyanide. A cyanide-stable respiration, catalyzed by formed CN-compounds, develops, however, after the initial inhibition. These findings confirm Lindahl's (1939, 1940 and 1941) results, and are in full concordance with his opinion that the cyanide-resistant respiration develops under the influence of the cyanide and that it has nothing to do with normal respiration. Robbie's findings show that the respiration of the unfertilized egg must proceed over iron porphyrins in the same manner as does that of the fertilized egg. Runnström (1930) had previously expressed the same opinion.

Robbie's results show conclusively that the respiration is of principally the same type both before and after fertilization. Thus it is understandable that temperature characteristics of respiration are found, as in the Psammechinus and Strongylocentrotus cases, that are the same before and after fertilization. On the other hand it is harder to understand how Q_{10} can differ so widely before and after fertilization as it does in Arbacia. It may be that the respiratory system operating in the unfertilized Arbacia egg is somehow unlike that of the just mentioned species. Any fundamental respiration pattern differences between the two classes of unfertilized sea-urchin eggs have, however, not been found.

Ballentine (1940) thinks that the oxidation in the sea-urchin egg of dimethyl-p-phenylenediamine requires cytochrome c as a mediator to the echinoderm oxidase. If so, cytochrome c must already be available and ready to function in the unfertilized egg, since Runnström (1932) and Örström (1932) found that dimethyl-p-phenylenediamine was oxidized at the same rate in unfertilized and fertilized eggs. Borei and Renvall (1949) could, however, not find that cytochrome c is essential for the cellular oxidation of dimethyl-p-phenylenediamine. Furthermore, hydroquinone, which can be oxidized by cytochrome oxidase (Keilin and Hartree, 1938) or echinoderm oxidase (Krahl and co-workers, 1941; Borei, 1945, Chapter IV: C1) only in the presence of a suitable mediator, has been shown by Runnström (1932) to be utilized in the unfertilized egg at a lower rate than in the fertilized one. This fact could possibly be taken as an indication that the carrier oxidized by the echinoderm oxidase has a lower concentration (or is less active or accessible) in the unfertilized egg than in the fertilized.

Many authors have doubted the existence of cytochrome c in the egg, as it has never been possible to find the cytochrome bands (cf. Krahl and co-workers, 1941). Thus Korr (1939), who originally (1937) thought that cytochrome c was released at fertilization, and Ballentine (1940) suppose that some other link, situated nearer the substrate, is put into operation at fertilization. It is, however, not unlikely that cytochrome c may have a sufficient carrier capacity in the sea-urchin egg, and yet not have a concentration high enough to permit spectroscopic detection. It may be pointed out (a) that the sea-urchin egg has a comparatively low Q_{O_2}, and (b) that cytochrome c is far more catalytically effective when attached to the proper intracellular protein particles than when working in solution. It may also be that another carrier has the same function in respect to echinoderm oxidase as has cytochrome c to cytochrome oxidase. (Concerning the differences between echinoderm oxidase and cytochrome oxidase, cf. Borei, 1945, and Krahl and co-workers, 1941).

The surplus in respiration induced by fertilization is either merely an addition to the respiration of the unfertilized egg, or caused by the fact that the induced respira-

tion might compete with and depress the latter. The smooth increase in post-fertilization respiration from the level of the unfertilized egg in Asterias speaks in favor of the addition possibility (cf. Borei, 1948a and Borei and Lybing, 1949), provided the respiratory mechanisms in eggs of starfishes and sea-urchins may be freely compared. The facts concerning temperature characteristics of oxygen consumption rates revealed in Arbacia, do not in themselves distinguish between the two possibilities, nor do any facts gained in this investigation concerning Psammechinus material. Concerning the declining pre-fertilization respiration see, however, Borei (1949), where it is shown that this respiration part does not influence post-fertilization respiration.

Parallels have been drawn between the respiration of the unfertilized sea-urchin egg and the diapause egg of the grasshopper. In respect to temperature characteristics, the grasshopper diapause egg has a very low Q_{10} in comparison with that of the active stages (Bodine and Evans, 1932), which is in contrast to the state in the sea-urchin egg. Too much stress may thus not be laid on such comparisons.

Summary

With Cartesian diver micro-respiration technique the temperature characteristics of the respiration of *Psammechinus miliaris* eggs were investigated:

1. Between 10 and 21° C. the gradual rise in oxygen consumption rate of the unfertilized egg is best represented by Krogh's standard curve. Q_{10} at 18° C. is around 2.5, at 10° C. around 3.5.
2. Fertilized eggs have the same temperature characteristics as unfertilized ones.
3. In respect to temperature characteristics just before and just after fertilization, two classes are distinguishable among the sea-urchins: The Strongylocentrotus-Psammechinus group with equal Q_{10} values before and after fertilization, and the Arbacia group with higher Q_{10} values before fertilization.
4. The significance of temperature characteristics for the biochemical processes involved in respiration of eggs before and after fertilization is discussed.

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